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Synthesis of Amino Acid Analogues of 5*H*-Dibenz[*b,f*]azepine and Evaluation of their Radical Scavenging Activity

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Abstract: A method for the synthesis of tyrosine, phenyl alanine, hydroxy proline and threonine free amino acid analogues of 5*H*-dibenz[*b,f*]azepine is proposed. 5*H*-dibenz[*b,f*]azepine was prepared by known method. The key intermediate 3-chloro-1-(5*H*-dibenz[*b,f*]azepine-5-yl)propan-1-one was obtained by *N*-acylation of 5*H*-dibenz[*b,f*]azepine with 3-chloro propionyl chloride. Further coupling of respective free amino acid to produce 2-(3-(5*H*-dibenz[*b,f*]azepine-5-yl)-3-oxopropylamino)3-(4 hydroxyphenyl) propanoic acid, 2-(3-(5*H*-dibenz[*b,f*]azepine-5-yl)-3-oxopropylamino)-3-phenyl propanoicacid,1-(3-(5*H*-dibenz[*b,f*] azepine-5-yl)-3-oxopropyl)-3-hydroxypyrolidine-2-carboxylic acid and 2-(3-(5*H*-dibenz[*b,f*] azepine-yl)-3-oxopropyl amino)-3-hydroxy butanoic acid. The synthesized compounds were evaluated for their potential over 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical scavenging activity. Butylated hydroxy anisole (BHA) and ascorbic acid (AA) were used as the reference antioxidant compounds and also the comparative study with synthesized compounds was done. Under our experimental conditions tyrosine, hydroxy proline and threonine analogues possess a direct scavenging effect on trapping the stable free radical DPPH. Hydroxy proline analogues showed a significant radical scavenging activity among the synthesized analogues.

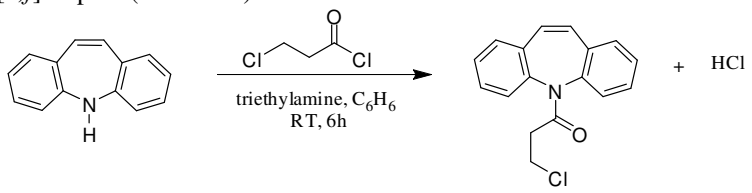
Keywords: 5*H*-dibenz[*b,f*]azepine, 3-Chloro-1-(5*H*-dibenz[*b,f*]azepine-5-yl) propan-1-one, Amino acid, Radical scavenging activity.

Introduction

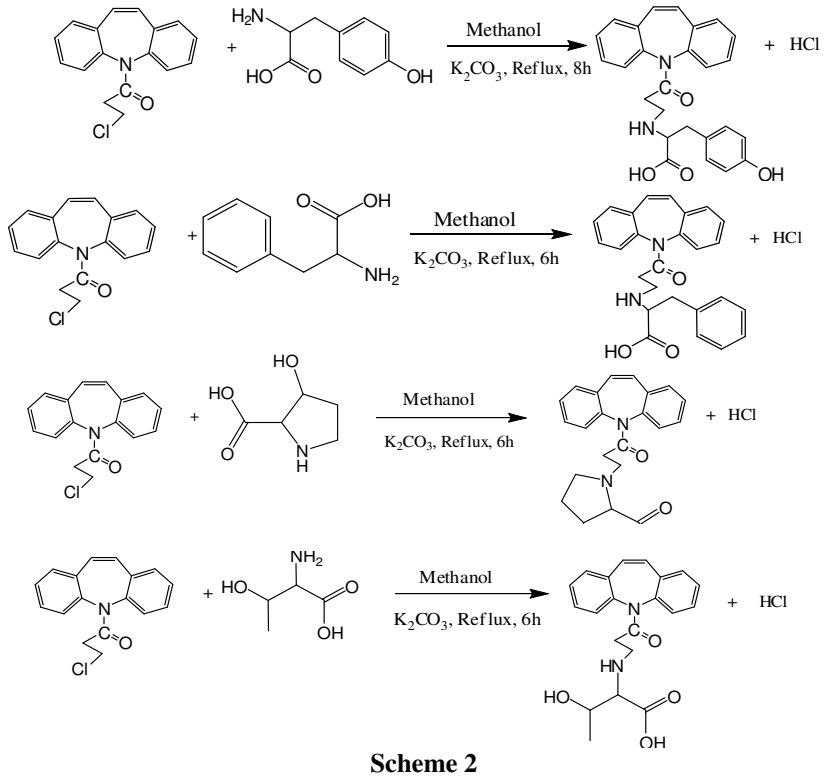
Free radicals can have a noxious effect on cells and it is believed that free radical damage is involved in the etiology of several diseases. The radicals are a by-product of various endogenous processes that can be stimulated by external factors, such as irradiation and xenobiotics[1]. Antioxidants protect against these radicals, and it is important to balance an enhanced radical production with a sufficient supply of antioxidants. There are two basic categories of antioxidants, namely, synthetic and natural. The most common synthetic antioxidants used in foods are compounds with phenolic structures of various degrees of substitution, whereas natural antioxidants are primarily plant phenolics and polyphenolic compounds that may occur in all the parts of plants[2]. Most of the antioxidants in use commercially *e.g*., butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT) and propyl gallate (PG) are synthetic[3]. Considerable progress has been made in recent years relating free radicals, especially reactive oxygen species in living cells to pathogenecity of various diseases[4-5] including hepatic and vascular diseases[6-7]. Efforts to discover antioxidants as useful drug candidates to combat these diseases are going on relentlessly. Numerous natural or synthetic antioxidant compounds have been tested with success in various disease models as well as in clinics[8]. Antioxidants are now forged as the drug candidate to combat these diseases. In the literature some tricyclic amines and their chemical structure shows antioxidant neuroprotective activity *in vitro*[9]. 5*H*-dibenz[*b*,*f*]azepine *i.e*., iminostilbene is a common a basic fused tricyclic amine. It is used as an intermediate for the synthesis of the registered anticonvulsant drug oxcarbazepine[10]. The structure of which has recently been reported[11]. Dibenz[*b*,*f*]azepine and its derivatives has been variously reported as having antiallergic activity, specifically antihistaminic activity, spansmolytic, serotonin antagonistic, anticonvulsive, antiemetic, antiepileptic, anti inflammatory, sedative and fungicidal action[12]. As a part of a series of studies into activity aspects, the radical scavenging activity of amino acid analogues of 5*H*-dibenz[*b*,*f*]azepine was determined and is reported here. The research on free radicals provides theoretical information for the medicinal development, and supplies some *in vitro* methods for quick optimizing drugs, it attracts more scientific attention from bioorganic and medicinal chemists. In addition to the traditional O-H bond type antioxidant, tricyclic amines having N-H bond functions as the antioxidant have attracted much research attention because aromatic amines (Ar_2NHs) have always been the central structure in many currently used drugs[13]. From the literature amino acids and some of their derivatives were also found to have antioxidant activity[14].

In the present paper we have used a model compound 3-chloro-1-(5*H*-dibenz[*b*,*f*] azepine-5-yl) propan-1-one to verify the possibility of obtaining the amino acid analogues of 5*H*-dibenz[*b*,*f*]azepine. Since their structure may justify a possible intervention on free radical process we have selected some of the free amino acids to explore better the chemistry and biological activities. The amino acid analogues were synthesized and their structure was established by chemical and spectral analysis. The compounds were investigated for *in vitro* DPPH free radical scavenging potential and the activity was compared to commercially available synthetic antioxidants namely butylated hydroxy anisole (BHA) and ascorbic acid (AA). A number of methods are available for the determination of free radical scavenging activity but the assay employing the stable 1, 1-diphenyl picryl hydrazyl radical (DPPH) has received most attention owing to its ease of use and its convenience[15]. This assay is the most widely used *in vitro* test through which to assess free radical scavenger capacities[16]. These studies may reflect the possibility for therapeutic uses and as a source of synthetic antioxidants.

5*H*-Dibenz[*b*,*f*]azepine was synthesized by applying known method[10]. The synthesis of amino acid analogues of 5*H*-dibenz[*b*,*f*]azepine was realized in two steps. First step the key intermediate 3-Chloro-1-5*H*-dibenz[*b*,*f*]azepine-5-yl) propan-1-one was prepared in good yield by *N*-acylation of 5*H*-dibenz[*b*,*f*]azepine with 3-chloro propionyl chloride in the presence of triethyl amine as base (Scheme 1). In the second step further coupling of respective amino acid to the intermediate were done to obtain the amino acid analogues of 5*H*-dibenz[*b*,*f*]azepine (Scheme 2).



Scheme 1



Scheme 2

Experimental

The following compounds and materials supplied by Sigma Aldrich were used: tyrosine, phenyl alanine, hydroxy proline, threonine. All the aminoacids used in the study were of L-configuration. Reagents and solvents like 3-chloro propionyl chloride, triethyl amine, benzene, methanol, chloroform, diethyl ether, acetic acid, ethyl acetate, sodium bicarbonate, anhydrous sodium sulphate were all of analytical grade and procured from Merck. TLC aluminium sheets silica gel 60 F_{254} was also purchased from Merck.

The IR spectra were recorded on a FT-IR021 model in KBr disc. The ^{1}H NMR spectra were recorded on Jeol GSX 400 MHz spectrophotometer using $CDCl_3$ as a solvent and the chemical shift (δ) are in ppm relative to internal standard. The mass spectra were recorded on Waters-Q-TOF Ultima spectrophotometer.

Synthesis of 3 -chloro -1-(5H-dibenz[b,f]azepine-5-yl) propan-1-one

To the well stirred solution of 5*H*- dibenz[*b,f*]azepine (2 mM) and triethyl amine (2.2 mM) in 50 mL benzene, 3- chloro propionyl chloride (2.2 mM) in 25 mL benzene was added drop by drop for about 30 min. Then the reaction mixture is stirred at room temperature for about 6 h. Progress of the reaction is monitored by TLC using 9:1 Hexane: Ethyl acetate mixture as mobile phase. After the completion of reaction, the reaction mass was quenched in ice cold water and extracted in diethyl ether. The ether layer was washed twice with 5% $NaHCO_3$ and once with distilled water. Finally the ether layer is dried with anhydrous Na_2SO_4. The light yellow crystal solid product was obtained by desolventation through rota vapour at 50 ^{0}C.

Light yellow solid, Yield (85%), M.p. 109-110^{0}C, IR (KBr): 3067.9 (Ar C-H), 1675.3 (C=O), 2971.0- 3026.1 (CH_2) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.8 (d, 2H, CH_2 – CO), 3.7 (d, 2H, CH_2Cl), 7.5 (m, 6H, Ar – H), 7.6 (d, 2H, C_4, C_6 of Ar – H), 6.9 (d, 2H, C_{10}, C_{12} of Ar – H); Mass (m/z % abundance): 284.56 (M^+, 98), 286.57 (35), 278.61 (2), 279.64 (2); Anal. Calcd. for $C_{17}H_{14}ClNO$: C, 71.96; H, 4.97; Cl, 12.49; N, 4.94; O, 5.64%; Found: C, 71.20; H, 4.88; Cl, 12.26; N, 4.79; O, 5.52%

Synthesis of 3-(4-hydroxyphenyl)-2-(3-(5H-dibenz[b,f]azepine-5-yl)-3-oxopropylamino) propanoic acid

Tyrosine (1.2 mM) in methanol (25 mL) was neutralized with triethyl amine (1.2 mM). To this, K_2CO_3 (600 mg) was added. Later the solution of 3-chloro-1-(5*H*-dibenz[*b,f*]azepine-5-yl) propan-1-one (1 mM) in methanol (50 mL) was added drop by drop for 30 min. The reaction mixture was refluxed for 6-8 h. The progress of the reaction mixture was monitored by TLC. The reaction mixture was then desolventized in rota vapour and the compound is extracted in ethyl acetate. The ethyl acetate layer was washed with water and dried over anhydrous Na_2SO_4. The light yellow solid was obtained by further desolventation in rota vapour at 50^{o}C.

Phenyl alanine, hydroxy proline and threonine amino acid analogues of 5*H*-dibenz [*b,f*]azepine were obtained by the same procedure. The analogues were separated and purified by column chromatography, using mixture of chloroform / methanol / acetic acid = 85: 15: 3. The products were characterized by IR, Mass ^{1}H NMR and Elemental Analysis.

Synthesis of 3-(4-hydroxyphenyl)-2-(3-(5H-dibenz[b,f]azepin-5-yl)-3-oxopropylamino) propanoic acid

Light yellow solid, Yield (72%), M.p. 91-93^{0}C, IR (KBr): 2467.0-3390.2 (OH-carboxylic acid), 3023.1 (Ar-C-H), 3316.5 (N-H), 1679.0 (C=O), 2812.0-2836.0 (CH_2) Cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.12 (d, 2H, CH_2 – CO), 2.58 (d, 2H, CH_2-N), 3.2 - 3.5 (m, 2H, benzylic CH_2 & t, 1H, CH – N), 6.8 (s, 2H, Ar – H, ortho to OH of tyrosine), 7.0 (s, 2H, C_{10}, C_{11} of Ar – H), 7.1 (s, 2H, Ar – H, para to OH of tyrosine), 7.35 – 7.54 (m, 8H, Ar – H), 9.5 (s, 1H, OH of tyrosine), 11.5 (s, 1H, carboxylic OH), 2.0 (s, 1H, NH); Mass (*m/z* % abundance): 427.84 (M^+ 97), 424.10 (50), 421.21 (2), 419.28 (35). Anal. Calcd. for $C_{26}H_{24}N_2O_4$: C, 72.88; H, 5.65; N, 6.54; O, 14.94%; Found: C, 72.12; H, 5.49; N, 6.78; O, 14.01%.

2-(3-(5H-dibenz[b,f]azepine-5-yl)-3-oxopropylamino)-3-phenyl propanoicacid

Light yellow solid, Yield (73%), M.p. 84-86^{0}C, IR (KBr): 2572.6-3191.5 (OH-carboxylic acid), 3021.9 (Ar-C-H), 3317.8 (N-H), 1678.9 (C=O), 2884.7-2918.0 (CH_2) Cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.12 (d, 2H, CH_2 – CO), 2.58 (d, 2H, CH_2-N), 3.2 - 3.5 (m, 2H, benzylic CH_2 & t, 1H, CH – N), 7.0 (s, 2H, C_{10}, C_{11} of Ar – H), 7.35 – 7.54 (m, 13H, Ar – H), 11.5 (s, 1H, carboxylic OH), 2.0 (s, 1H, NH);

Mass (*m/z* % abundance): 409.14 (M^+ 98), 410.14 (9), 413.14 (3); Anal. Calcd. for $C_{26}H_{24}N_2O_3$: C, 75.71; H, 5.86; N, 6.79; O, 11.64%; Found: C, 75.54; H, 6.12; N, 6.42; O, 11.99%.

1-(3-(5H-dibenz[b,f]azepine-5-yl)-3-oxopropyl)-3-hydroxypyrolidine-2-carboxylic acid

Light yellow solid, Yield (74%), M.p. 82-84^{0}C, IR (KBr): 2572.0-3261.6 (OH-carboxylic acid), 3021.3 (Ar-C-H), 3320.1 (N-H), 1669.9 (C=O), 2895.9(CH_2) Cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.0 - 2.5 (t, 2H, CH_2CO, and m, 2H, CH_2N), 2.58 (d, 2H, CH_2 – N of pyrolidine), 1.7 (d, 2H, CH_2 of pyrolidine), 4.80 (s, 1H, OH of pyrolidine), 3.8 (m, 1H, CH-OH), 3.1 (d, 1H, CH of carboxylic acid), 11.5 (s, 1H, carboxylic OH), 7.35 – 7.54 (m, 8H, Ar – H), 7.0 (s, 2H, C_{10}, C_{11} of Ar – H); Mass (*m/z* % abundance): 378.23 (M^+ 92), 381.22 (3), 392.25 (18), 393.26 (2); Anal. Calcd. for $C_{22}H_{22}N_2O_4$: C, 69.83; H, 5.86; N, 7.40; O, 16.91% ; Found: C, 70.23; H, 6.17; N, 7.28; O, 16.88%.

2-(3-(5H-dibenz[b,f]azepine-yl)-3-oxopropyl amino)-3-hydroxy butanoic acid

Light yellow solid, Yield (78%), M.p. 79-81^{o}C, IR (KBr): 2617.6-3316.8 (OH-carboxylic acid), 3022.2 (Ar-C-H), 3361.4 (N-H), 1667.3 (C=O), 3022.2 (CH_2) Cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.12 (d, 2H, CH_2CO), 2.6 (d, 2H, CH_2N), 2.0 (s, 1H, NH), 3.5 (d, 1H, C H - N), 3.7 (t, 1H, CH – OH), 5.0 (s, 1H, OH), 1.25 (d, 1H, CH_3), 11.5 (s, 1H, COOH), 7.35 – 7.81 (m, 8H, Ar – H), 7.0 (s, 2H, C_{10}, C_{11} of Ar – H); Mass (*m/z* % abundance): 366.92 (M^+ 92), 365.21 (32), 368.01 (18), 393.26 (2); Anal. Calcd. for $C_{21}H_{22}N_2O_4$: C, 68.84; H, 6.05; N, 7.65; O, 17.47% ; Found: C, 68.34; H, 6.18; N, 7.83; O, 17.21%.

Radical scavenging activity

In the present study, the synthesized compounds were screened for their DPPH free radical scavenging activity. The compounds were dissolved in distilled ethanol (50 mL) to prepare 1000 µM solution. Solutions of different concentrations (10 µM, 50 µM, 100 µM, 200 µM and 500 µM) were prepared by serial dilution and the free radical scavenging activity was studied.

DPPH radical scavenging activity

The DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging effect was carried out according to the method first employed by Blois[17]. Compounds of different concentrations were prepared in distilled ethanol, 1mL of each compound solutions having different concentrations (10 µM, 50 µM, 100 µM, 200 µM and 500 µM) were taken in different test tubes, 4 mL of a 0.1 mM ethanol solution of DPPH was added and shaken vigorously. The tubes were then incubated in the dark room at RT for 20 min. A DPPH blank was prepared without compound, and ethanol was used for the baseline correction. Changes (decrease) in the absorbance at 517 nm were measured using a UV-visible spectrophotometer and the remaining DPPH was calculated. The percent decrease in the absorbance was recorded for each concentration, and percent quenching of DPPH was calculated on the basis of the observed decreased in absorbance of the radical. The radical scavenging activities were expressed as the inhibition percentage and were calculated using the formula:

$$\text{Radical scavenging activity (\%)} = [(A_0 - A_1 / A_0) \times 100]$$

Where A_0 was the absorbance of the control (blank, without compound) and A_1 was the absorbance of the compound. The radical scavenging activity of BHA and ascorbic acid was also measured and compared with that of the different synthesized compound. For all the compounds and standards half inhibition concentration (IC_{50}) was calculated and showed in the Table 1.

Results and Discussion

The starting material 3-Chloro-1-(5*H*-dibenz[*b,f*]azepine-5-yl) propan-1-one was prepared in good yield by *N*-acylation reaction. In practice, 5*H*-dibenz[*b,f]*azepine and 3-chloro propionyl chloride were mixed in 1:1.2 ratio in the presence of triethylamine as base. The reaction was carried out for 6 h at room temperature. The final product was separated from the reaction mixture by washing HCl salt (precipitate) with distilled water three times, and then the compound was extracted with diethyl ether. The ether layer was washed with 5% $NaHCO_3$ for the removal of the remaining quantity of acids. The organic layer was separated. The solvent was removed by vacuum -distillation and finally product was isolated from coloum chromatography by using 9:1 hexane and ethyl acetate.

Amino acid analogues of 5*H*- dibenz[*b,f*]azepine were obtained by mixing respective amino acid: 3-Chloro-1-(5*H*-dibenz[*b,f*]azepine-5-yl) propan-1-one = 1.2 mM: 1 mM. The free amino acid was made dissolved in methanol by adding 1-2 mL of triethyl amine, then 600 mg of anhydrous K_2CO_3 was added and stirred for 30 min. 3-Chloro-1-(5*H*-dibenz[*b,f*] azepine-5-yl) propan-1-one in methanol was added drop by drop and refluxed for 6 h. The final product was separated from the mixture by desolventation of methanol by vacuum - distillation and then the product was extracted with ethyl acetate. Ethyl acetate layer was washed with water to remove K_2CO_3 and dried with anhydrous Na_2SO_4. Further desolventation by vacuum distillation was done and the final product was isolated from column chromatography by using chloroform / methanol / acetic acid = 85: 15: 3 as mobile phase.

The synthesized compounds were screened for their DPPH free radical scavenging activity. The DPPH test provided information about the reactivity of the tested compounds with a stable free radical. Because of its odd electron, the DPPH radical showed a strong absorption band at 517 nm in visible spectroscopy (a deep purple color). As this electron is paired off in the presence of a free radical scavenger, absorbance vanishes and the resulting discoloration is stoichiometric with respect to the number of electrons taken up. This bleaching of DPPH absorption, which occurs in the odd electron of the radical is paired, is thus representative of the capacity of the compounds to scavenge free radicals independently. The DPPH free radical scavenging ability for the synthesized compounds is showed in the Figure 1. The IC_{50} or inhibition concentration (IC_{50}) was obtained from the graph of the percentage of scavenging radical versus the concentration of antioxidant[18-19]. Half inhibition concentration (IC_{50}) for all the compounds and standards is summarized in the Table 1.

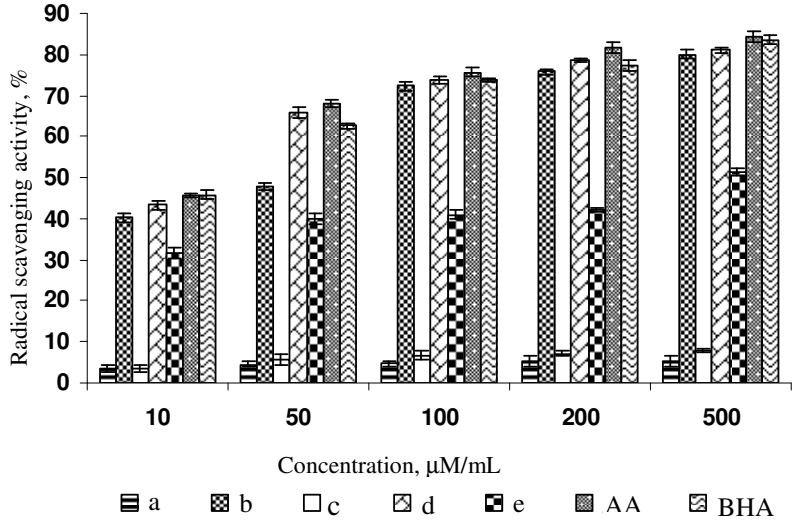
Initially, the DPPH free radical scavenging capacity of 3-chloro-1-(5*H*-dibenz[*b,f*] azepine-5-yl) propan-1-one was assessed and found to be not effective. Consequently, molecules with amino acid groups were coupled by elimination reaction to enhance the radical scavenging activity effect. Further, the test performed with compounds bearing amino acid substituent in the chloride position. Tyrosine, hydroxy proline and threonine amino acid analogues are having radical scavenging activity with a major activity for hydroxy proline analogues. When tyrosine molecule is coupled the activity will be enhanced due to the presence of phenolic group. In the case of phenyl alanine, activity disappears due to the absence of –OH group in the aromatic ring.

When hydroxy proline is made coupled the RSA increases with a major activity due to the presence of –OH group attached to the five member heterocyclic ring *i.e*., pyrolidine ring. Presence of free –OH group in threonine showed the average activity among all the synthesized analogues. These results show the major importance of the amino acid substituent in DPPH free radical effects. Increasing order of DPPH activity for the synthesized compounds can be classified as **d**. 1-(3-(5*H*-dibenz[*b*,*f*]azepine-5-yl)-3-oxopropyl)-3hydroxypyrolidine-2-carboxylicacid **> b** 2-(3-(5*H*-dibenz[*b*,*f*]azepine-5-yl)-3-oxopropylamino)-3-(4-hydroxyphenyl) propanoic acid > **e**. 2-(3-(5*H*-dibenz[*b*,*f*]azepine-yl)-3-oxopropyl amino)-3-hydroxy butanoic acid > **c**. 2-(3-(5*H*-dibenz[*b*,*f*]azepine-5-yl)-3-oxopropylamino)-3phenylpropanoic acid > **a**. 3-chloro-1-(5*H*-dibenz[*b*,*f*]azepine-5-yl) propan-1-one.

Table 1. 50% Inhibition of DPPH radical by the analogues of dibenz[*b*,*f*]azepine.

Compound	IC_{50} µM/mL
Compound a	-
Compound b	42.17 ± 1.18
Compound c	-
Compound d	3.01 ± 0.74
Compound e	163.59 ± 1.19
AA	4.94 ± 0.63
BHA	5.26 ± 0.87

IC_{50} : Concentration required for 50% reduction of 0.1 mM DPPH radical.
Values represent means ± SD (n=3).
where '–'corresponds to negligible activity.



Where; *a. 3-chloro-1-(5H-dibenz[b,f]azepine-5-yl) propan-1-one*
b. 2-(3-(5H-dibenz[b,f]azepine-5-yl)-3-oxopropylamino)-3-(4-hydroxyphenyl) propanoic acid,
c. 2-(3-(5H-dibenz[b,f]azepine-5-yl)-3-oxopropylamino)-3-phenylpropanoic acid,
d. 1-(3-(5H-dibenz[b,f]azepine-5-yl)-3-oxopropyl)-3-hydroxypyrolidine-2-carboxylic acid,
e. 2-(3-(5H-dibenz[b,f]azepine-yl)-3-oxopropyl amino)-3-hydroxy butanoic acid,
AA. Ascorbic acid, and BHA. Butylated Hydroxy Anisole.

Figure 1. DPPH free radical scavenging ability for 3-chloro-1-(*5H*-dibenz[*b*,*f*]azepine-5-yl) propan-1-one and its amino acid analogues. Values represent means ± SD (n = 3).

The RSA activity of synthesized compounds was compared to internal standards (AA and BHA). Amonge the synthsised compounds tyrosine and hydroxy proline showed more effective DPPH activity but slightly less than the standards. It was observed that the RSA activity (%) of all the synthesized compounds increase with increase in concentrations.

Conclusion

The method proposed by us reproduces the synthesis of amino acid analogues of 5*H*-dibenz [*b*,*f*]azepine. The synthesized compounds were characterized with the help of spectroscopic techniques and were screened for their ability to DPPH radical scavenging activity. Based on the biological assay it was found that the moiety of 3-chloro-1-(5*H*-dibenz[*b*,*f*]azepine-5-yl) propan-1-one containing tyrosine, hydroxy proline and threonine showed promising DPPH radical scavenging activity. But hydroxy proline analogues were found to be most effective radical scavenging activity than the other analogues but slightly less than the internal standards (AA and BHA). Our study provides evidence that several amino acid analogues of 5*H*-dibenz[*b*,*f*]azepine exhibit interesting direct DPPH free radical scavenging activity. These effects may be useful in the treatment of pathologies in which free radical oxidation plays a fundamental role.

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